

**STRUCTURAL ELUCIDATION OF POLYMERIC DITERPENOIDS
IN FOSSIL GYMNOSPERM RESINS
BY MEANS OF PYROLYSIS COMBINED WITH GC-MS.**

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Keywords: fossil gymnosperm resins, polymeric diterpenoids, flash-pyrolysis-gas chromatography-mass spectrometry.

INTRODUCTION

Fossil resins originating from Gymnosperm trees have been the subject of several studies concerning the structure of their high molecular weight fractions ¹⁻⁴. Although such resins may be sourced from quite different plant families the structures of the macromolecular moieties have similar features. Formation of these substances is thought to occur *via* a light and/or oxygen induced polymerisation of diterpenoid monomers with a labdane carbon skeleton, such as *communica* acid ¹. Because the polymers are insoluble in common organic solvents, structural studies have been performed using techniques like IR ⁵, solid state NMR ^{3,6,7} and Py-MS ^{4,8}. In this study we show that Curie point pyrolysis-GC-MS and off-line pyrolysis GC-MS are useful methods to gain information on the structure of the polymeric fractions of fossil resins. For this purpose a fossil resin from the Fushun coal mine in China was analyzed.

EXPERIMENTAL

Curie-point pyrolysis-gas chromatography-flame ionisation detection (Py-GC-FID) analyses were performed with a Hewlett-Packard 5890 gas chromatograph using a FOM-3LX unit for pyrolysis. The samples were applied to a ferromagnetic wire with Curie temperatures of 770°C (Fe) or 358°C (Ni). The gas chromatograph, equipped with a cryogenic unit, was programmed from 0°C (5 min) to 320°C (20 min) at a rate of 3°C/min. Separation was achieved using a fused-silica capillary column (25m x 0.32mm) coated with CP Sil-5 (film thickness 0.4 µm). Helium was used as the carrier gas.

Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) analyses were performed using the same equipment and conditions as described above for Py-GC-FID connected with a VG 70S mass spectrometer operated at 70 eV with a mass range *m/z* 40-800 and a cycle time of 1.8 s.

Extraction of the resin was achieved by thoroughly mixing the finely powdered resin ultrasonically several times with a variety of solvents like methanol, dichloromethane, ethylacetate and acetone. The combined extracts were methylated with diazomethane and analysed by GC-MS using the same equipment described for Py-GC-MS (CP Sil-5 column, film thickness 0.32 µm, temperature program from 70°C to 320°C (20 min) at a rate of 4°C/min).

For off-line pyrolysis-gas chromatography-mass spectrometry, an aliquot of the extracted resin was transferred into a quartz tube and heated under a constant flow of nitrogen (100 ml/min) at 425°C for 45 min. The released products were trapped in two flasks with pentane. The first was held at room temperature and the second at -20°C. The trapped pyrolysates were combined and analyzed by GC-MS as described above.

RESULTS AND DISCUSSION

Extraction of resins is often incomplete because of inclusion of soluble compounds in the insoluble macromolecular matrix. To differentiate between pyrolysis products and evaporated compounds the whole resin was analysed at both a Curie temperature of 770°C and of 358°C. At 358°C no pyrolysis takes place and only volatile compounds are analysed. The chromatograms of both analyses of the whole resin are shown in Figures 1a and 1b. The compounds released were tentatively identified by Py-GC-MS. The most abundant compound present in the mixture obtained at a Curie temperature of 770°C is a mono-unsaturated bicyclic C₁₅-carboxylic acid. The mass spectrum and suggested structure for this compound are shown in Figure 2a. Furthermore, a series of C₆, C₇ and C₈ unsaturated branched acyclic hydrocarbons is present.

Also very abundant are camphor and dehydroabietane. Comparison of the product mixtures obtained at a Curie temperature of 770°C (Fig. 1a) with that of the flash-evaporate (Fig. 1b) shows that both camphor and dehydroabietane are present as such in the resin. This is also true for the C_{15} -acid. However, at a Curie temperature of 770°C the latter compound is much more abundant, indicating that it is formed upon pyrolysis as well. This is also supported by the presence of a co-eluting similar compound with an additional double bond, which is absent in the flash-evaporate.

To concentrate on the products formed upon pyrolysis, and thus on the structure of the insoluble macromolecule, the resin was thoroughly extracted and the residue was pyrolysed at a Curie temperature of 770°C. The resulting chromatogram is shown in Figure 3a. Compounds like camphor and dehydroabietane are no longer present, showing that the extraction was successful. Flash evaporation at a Curie temperature of 358°C of the residue showed that virtually all the volatile compounds were extracted indeed. From the Py-GC analysis it is clear that the major pyrolysis products are the bicyclic C_{15} -acid, a similar bicyclic C_{14} -acid and the series of branched alkenes. Comparison of the chromatogram of the flash-evaporate (Fig. 1b) with that of the methylated extract (Fig. 3b) shows that many diterpenoid methyl esters with an abietane or pimarane carbon skeleton are present in the extract, but not in the evaporate. These C_{20} -compounds are probably present as free acids in the evaporate and hence not GC-amenable on the apolar column used. This is exemplified by the broadened peak reflecting the C_{15} -carboxylic acid in the evaporate. It should be noted that no bicyclic C_{20} -diterpenoids with labdane carbon skeletons could be detected in the pyrolysate nor in the extract. At the end of the chromatogram of the extract a broad hump is visible. Mass spectrometric analysis showed that this hump reflects several badly resolved dimeric diterpenoid acids, probably with labdane carbon skeletons. The mass spectrum of one of these compounds and a possible structure are shown in Figure 2b.

The chromatogram of the GC analysis of the methylated pyrolysate obtained by off-line pyrolysis of the extracted resin is shown in Figure 4. The pyrolysate obtained by off-line pyrolysis resembles the pyrolysate obtained by flash pyrolysis very well. The most abundant compounds are the mono- and disaturated bicyclic C_{15} methyl esters. Again no bicyclic C_{20} diterpenoid methyl esters were detected.

The results shown indicate that the fossil resin consists of two fractions. A soluble low-molecular-weight fraction and an insoluble high-molecular-weight fraction. The distinction between these two fractions is however not clear-cut, as shown by the presence of dimeric diterpenoids in the extract. The flash-evaporate obtained at a Curie-temperature of 358°C can however not be taken as representative for the soluble fraction, because of the presence of non GC-amenable acids, which will do show up in the chromatogram. The high-molecular-weight fraction yields upon pyrolysis predominantly an unsaturated bicyclic C_{15} -carboxylic acid, which is probably the pyrolysis product of a polymeric diterpenoid. As shown in Scheme 1 a pyrolysis mechanism can be conceived starting from a polymeric structure as suggested in literature ¹⁻⁴. This mechanism can also explain the presence of unsaturated branched hydrocarbons in the pyrolysate. The acid is also found in the methylated extract as its methyl ester, indicating that some thermal breakdown of the polymer has already occurred in nature. On the other hand, volatile compounds like camphor are still included in the resin, indicating that the resin has not undergone severe diagenesis. No bicyclic C_{20} -compounds are formed upon pyrolysis of the polymer, as was shown by the off-line pyrolysis. Conclusions about the structure of the polymer in Gymnosperm resins based on the presence of diterpenoid monomers in pyrolysates should therefore be taken with care ^{4,8}, because they are probably based on included, soluble and volatile compounds and not on pyrolysis products. Because no methyl esters of the C_{15} -pyrolysis products were detected in the flash pyrolysate, the polymer of the fossil resin used in this study must be built up of non-methylated monomers. The question recently discussed in the literature by several authors ^{7,8}, as to whether or not Yallourn resinite polymer is methylated for a significant part, can therefore quickly be answered using the analytical methods described above.

CONCLUSIONS

The results described above support earlier conclusions about the structure of polymers present in fossil and recent gymnosperm resins. The resin studied contains a polymerized bicyclic diterpenoid, probably communic acid. Polymerization has occurred through the side chain, possibly via a 1,2 mechanism. The methods used in this work are relatively fast and simple and the results are complementary to those obtained by spectroscopic methods. Furthermore, they allow for further and more detailed studies of the basic structural units present in such polymers and the way they have polymerized by isolation and structural analysis of dimers and pyrolysis products. Such studies are now in progress.

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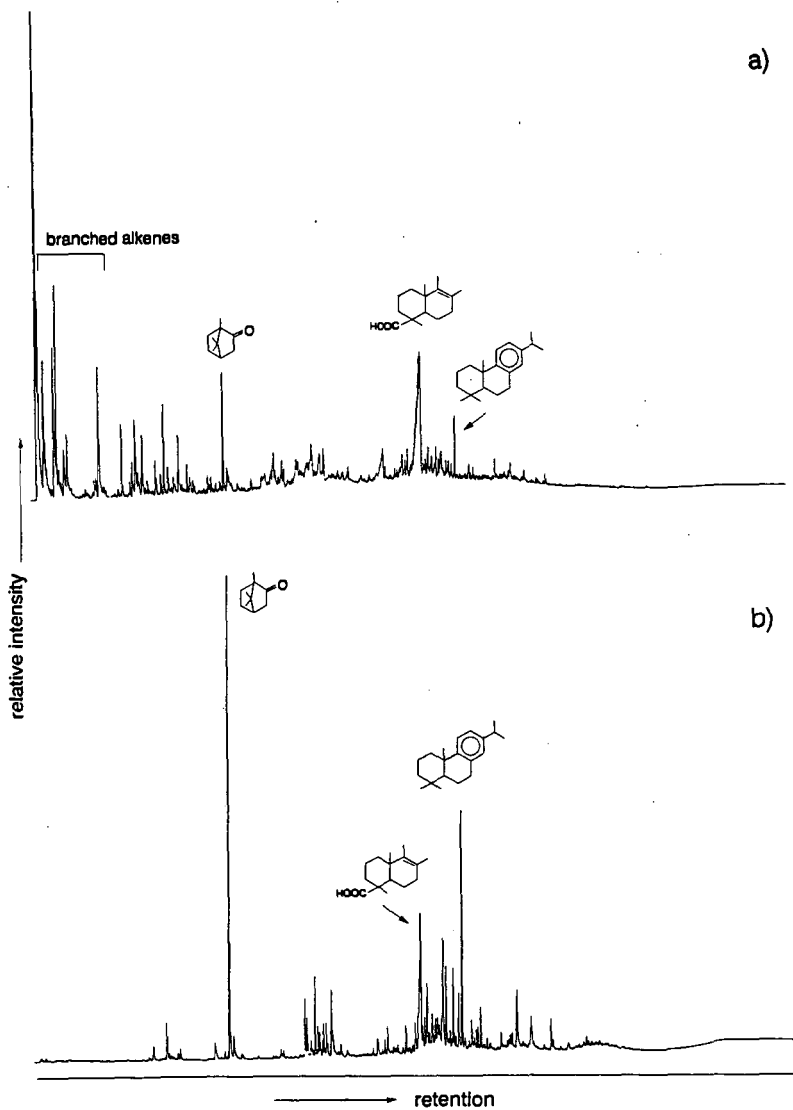


Fig. 1. Py-GC-FID traces of the whole fossil resin at Curie temperatures of a) 770°C and b) 358°C.

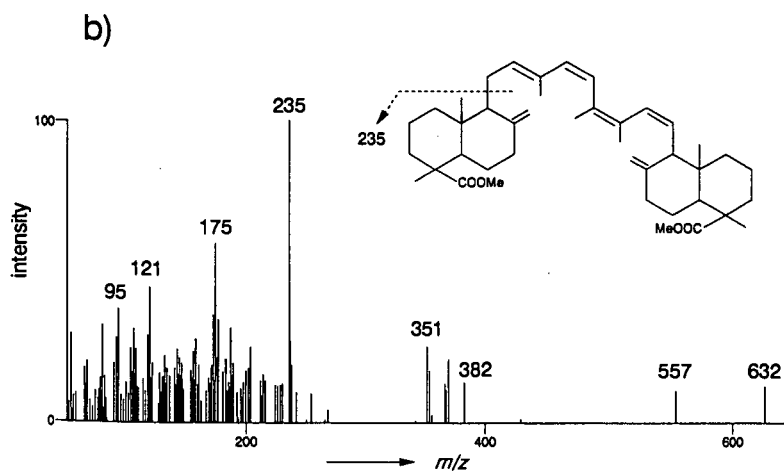
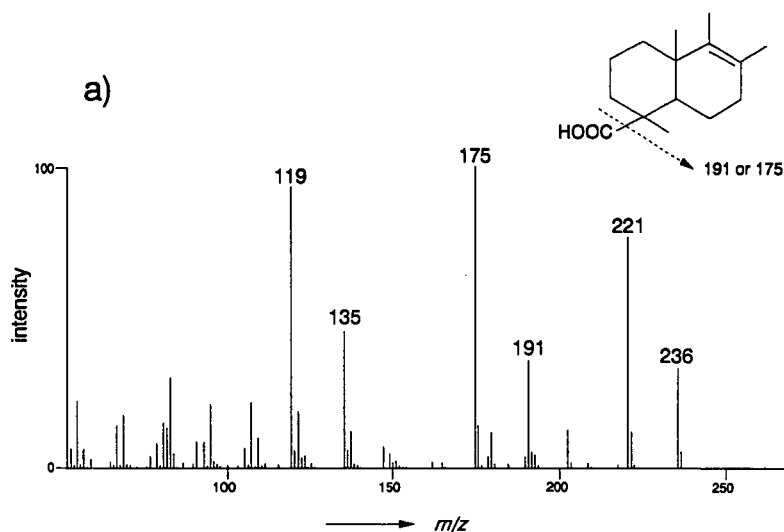


Fig. 2. Mass spectra of a) the major pyrolysis product of the fossil resin at a Curie temperature of 770°C and b) a diterpenoid dimer present in the resin extract.

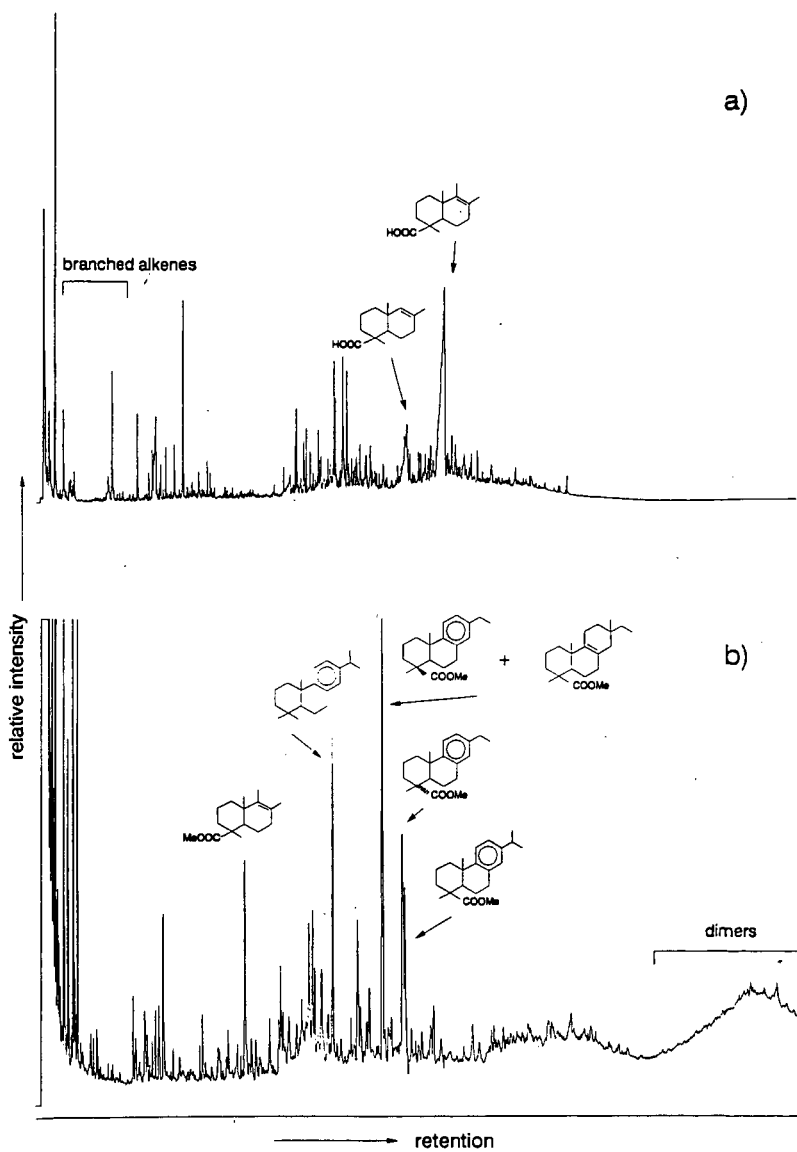


Fig. 3. a) Py-GC-FID trace of the extracted resin at a Curie temperature of 770°C. b) GC-FID trace of the methylated resin extract.

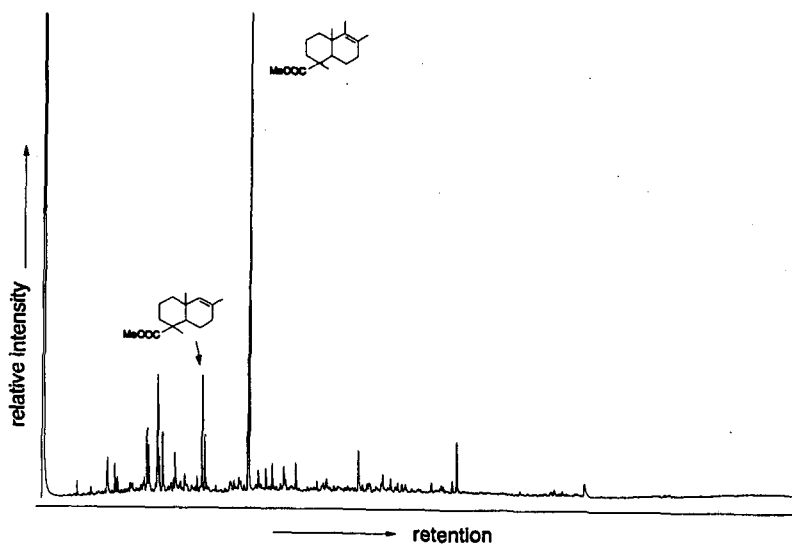


Fig. 4. GC-FID trace of the methylated pyrolysate obtained from the off-line pyrolysis of the extracted resin.

Scheme 1

